

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Nan, Guo-Ling
Nagai, Chifumi
- (ii) TITLE OF INVENTION: COMPOSITIONS AND METHODS FOR GENETIC TRANSFORMATION OF PINEAPPLE

(iii) NUMBER OF SEQUENCES: 6

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- (C) CITY: San Francisco
- (D) STATE: California
- (E) COUNTRY: United States of America
- (F) ZIP: 94104

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: US 09/078,862
- (B) FILING DATE: 14-MAY-1998
- (C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: Carroll, Peter G.
- (B) REGISTRATION NUMBER: 32,837
- (C) REFERENCE/DOCKET NUMBER: UH-03321

(ix) TELECOMMUNICATION INFORMATION:

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- (B) TELEFAX: (415) 397-8338

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2145 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGATCTACAA TTATCGCAAC GTGTTACACA TTTTGTGCTA CAATATAACCT TCACCATT	60
GTGTATATAT AAAGGTTGCA TCTCTTCAAA CAAAAATCAC TCCATCACAA CACAATGTCT	120
TCTTCTTCTT CTATTACTAC TACTCTTCCT TTATGCACCA ACAAATCCCT CTCTTCTTCC	180
TTCACCAACCA CCAACTCATC CTTGTTATCA AAACCCCTCTC AACTTTCCCT CCACGGAAAGG	240
CGTAATCAAA GTTTCAAGGT TTCATGCAAC GCAAACAAACG TTGACAAAAA CCCTGACGCT	300

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CCATTAGCGA CTGCTGCACC TATAACCACCT CCTGATCTCA AGTCTTGTGG TACTGCCCAT	420
GTAAAAGAAG GTGTTGATGT AATATACAGT TGTTGCCCTC CTGTACCCGA TGATATCGAT	480
AGTGTTCGCT ACTACAAGTT CCCTTCTATG ACTAAACTCC GCATCCGCC CCCTGCTCAT	540
GCGGCGGATG AGGAGTACGT AGCCAAGTAT CAATTGGCTA CGAGTCGAAT GAGGGAACCTT	600
GATAAAAGACC CCTTGACCC TCTTGGCTTT AAACAACAAG CTAATATTCA TTGTGCTTAT	660
TGCAACGGTG CTTACAAAGT TGGTGGCAA GAATTGCAAG TTCATTTCTC GTGGCTTTTC	720
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GATCCAACCTT TTGCTTTACC TTACTGGAAT TGGGATCATC CAAAAGGCAT GCGTATAACCT	840
CCCATGTTG ATCGTGAGGG ATCATCTCTT TACGATGAGA AACGTAACCA AAATCATCGC	900
AATGGAACTA TTATTGATCT TGGTCATTTT GGTAAAGGAAG TTGACACACC TCAGCTACAG	960
ATAATGACTA ATAATTTAAC CCTAATGTAC CGTCAAATGG TTACTAATGC TCCTTGCCCT	1020
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TTCAAGCTGG CGATAACTGA ACTGTTGGAG GATATTGGAT TGGAAAGATGA AGATACTATC	1800
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ATAGCTGATT GCTAGTTGTG TTAATGCTAT GTATGAAATA AATAAAATGGT TGTCTTCCAT	2040
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3509 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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GCCGGCGGCG	GCGGCGGCGG	GGGGGACCCC	AGGTCGCCGA	CGAAGGCGGC	GAGCCCCCGC	240
GGCGCGCAC	TGAACTTCAA	CCCCTCGCAC	TACTTCGTCG	AGGAGGTGGT	CAAGGGCGTC	300
GACGAGAGCG	ACCTCCACCG	GACGTGGATC	AAGGTCGTCG	CCACCCGCAA	CGCCCGCGAG	360
CGCAGCACCA	GGCTCGAGAA	CATGTGCTGG	CGGATCTGGC	ACCTCGCGCG	CAAGAAGAAG	420
CAGCTGGAGC	TGGAGGGCAT	CCAGAGAAC	TCGGCAAGAA	GGAAGGAACA	GGAGCAGGTG	480
CGTCGTGAGG	CGACGGAGGA	CCTGGCCGAG	GATCTGTCAG	AAGGCGAGAA	GGGAGACACC	540
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CATGGTCTTG	TTCGTGGAGA	AAACATGGAA	CTAGGTCGTG	ATTCTGATAC	AGGTGGCCAG	720
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GACCTCTTCA	CTCGTCAAGT	GTCATCTCCT	GACGTGGACT	GGAGCTACGG	TGAGCCAACC	840
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CACCGCTGGT CCCATGACGG CGCGAGGCAG ACCATAGCGA AGCTCATGGG CGCTCAGGAC	2820
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TATAAAATAA GTTGTGAACA GTACCGCGGG TGTGTATATA TATATTGCAG TGACAAATAA	3420
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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 13 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "DNA"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AATTGGGCAC GAG 1.3

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 9 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "DNA"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTCGTGCCG 9

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 623 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: circular
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "DNA"
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

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GTAACCACCC	CGCGTCCCTC	TCCTCTTCT	TTCTCCGTTT	TTTTTTCCG	TCTCGTCTCG	180
ATCTTGGCC	TTGGTAGTTT	GGGGCGAGA	GGCGGCTTCG	TCGCCCAGAT	CGGTGCGCGG	240
GAGGGCGGG	ATCTCGCGGC	TGGGTCTCGG	CGTGCGGCCG	GATCCTCGCG	GGGAATGGGG	300
CTCTCGGATG	TAGATCTGAT	CCGCCGTTGT	TGGGGGAGAT	GATGGGGCGT	TTAAAATTTC	360
GCCATGCTAA	ACAAGATCAG	GAAGAGGGGA	AAAGGGCACT	ATGGTTTATA	TTTTTATATA	420

TTTCTGCTGC TGCTCGTCAG GCTTAGATGT GCTAGATCTT TCTTTCTTCT TTTTGTGGGT	480
AGAATTTGAA TCCCTCAGCA TTGTTCATCG GTAGTTTTC TTTTCATGAT TTGTGACAAA	540
TGCAGCCTCG TGCGGAGCTT TTTTGTAGGT AGAAGATGGC TGACGCCGAG GATGGGGGAT	600
CCCCGGGTGG TCAGTCCCTT ATG	623

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ala Asp Ala Glu Asp Gly Gly Ser Pro Gly Gly Gln Ser Phe Met			
1	5	10	15

CLAIMS

1. A stably transformed transgenic pineapple-like totipotent body.
2. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body is a protocorm-like body.
3. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body is a callus.
4. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body comprises undifferentiated cells.
5. The stably transformed transgenic pineapple-like totipotent body of Claim 1, wherein said totipotent body is a pineapple totipotent body.
6. A stably transformed transgenic pineapple-like plant.
7. The stably transformed transgenic pineapple-like plant of Claim 6, wherein said plant is derived from a transgenic pineapple-like totipotent body.
8. The stably transformed transgenic pineapple-like plant of Claim 7, wherein said totipotent body is a protocorm-like body.
9. The stably transformed transgenic pineapple-like plant of Claim 7, wherein said totipotent body is a callus.
10. The stably transformed transgenic pineapple-like plant of Claim 6, wherein said plant is a pineapple plant.

11. A method for producing a stably transformed transgenic pineapple-like totipotent body, comprising:

- a) providing:
 - i) a pineapple-like totipotent body; and
 - ii) a heterologous nucleic acid comprising an oligonucleotide sequence of interest; and
- b) introducing said oligonucleotide sequence of interest into said pineapple-like totipotent body under conditions such that a stably transformed transgenic pineapple-like totipotent body is produced.

12. The method of Claim 11, wherein said pineapple-like totipotent body is a callus.

13. The method of Claim 11, wherein said pineapple-like totipotent body is a protocorm-like body.

14. The method of Claim 11, further comprising c) selecting said transgenic pineapple-like totipotent body.

15. The method of Claim 14, wherein said selecting is in liquid medium.

16. The method of Claim 14, wherein said selecting comprises detecting said oligonucleotide in the genome of said stable transformed pineapple-like totipotent body.

17. The method of Claim 11, wherein said pineapple-like totipotent body is a pineapple totipotent body.

18. The method of Claim 11, wherein said oligonucleotide is introduced by bombarding said pineapple-like totipotent body with said oligonucleotide sequence of interest.

19. The method of Claim 11, wherein said oligonucleotide is introduced by infecting said pineapple-like totipotent body with *Agrobacterium* comprising said oligonucleotide sequence of interest.

20. The method of Claim 19, wherein said infecting comprises microwounding said pineapple-like totipotent body to produce a microwounded totipotent body, and infecting said microwounded totipotent body with said *Agrobacterium*.
21. The method of Claim 19, wherein said *Agrobacterium* is agropine-type.
22. The method of Claim 19, wherein said *Agrobacterium* is nopaline-type.
23. The method of Claim 19, wherein said *Agrobacterium* is octopine-type.
24. A method for producing a stably transformed transgenic pineapple-like plant, comprising:
- providing:
 - a pineapple-like totipotent body; and
 - a heterologous nucleic acid comprising an oligonucleotide sequence of interest;
 - introducing said oligonucleotide sequence of interest into said pineapple-like totipotent body under conditions such that a transgenic pineapple-like totipotent body is produced; and
 - culturing said transgenic pineapple-like totipotent body under conditions such that a stably transformed transgenic pineapple-like plant is produced.
25. The method of claim 24, wherein said oligonucleotide is introduced by bombarding said pineapple-like totipotent body with said oligonucleotide sequence of interest.
26. The method of Claim 24, wherein said oligonucleotide is introduced by infecting said pineapple-like totipotent body with *Agrobacterium* comprising said oligonucleotide sequence of interest.
27. The method of Claim 24, further comprising prior to step c) selecting said stably transformed transgenic pineapple-like totipotent body.
28. The method of Claim 27, wherein said selecting is in liquid medium.

29. The method of Claim 27, wherein said selecting comprises detecting said oligonucleotide in the genome of said stably transformed transgenic pineapple-like totipotent body.
30. The method of Claim 24, wherein said pineapple-like plant is a pineapple plant.
31. The method of Claim 24, wherein said sequence of interest is selected from the group consisting of oligonucleotides encoding sucrose phosphate synthase, CpTi, thaumatin, and ACC deaminase.
32. The method of Claim 24, wherein said sequence of interest is selected from the group consisting of antisense polyphenol oxidase and ACC oxidase.
33. A method for producing a pineapple-like protocorm-like body comprising maintaining said pineapple-like protocorm-like body in liquid medium.
34. The method of Claim 33, wherein said liquid medium substantially comprises PI medium.
35. The method of Claim 33, wherein said pineapple-like protocorm-like body is a pineapple protocorm-like body.

Figure 1

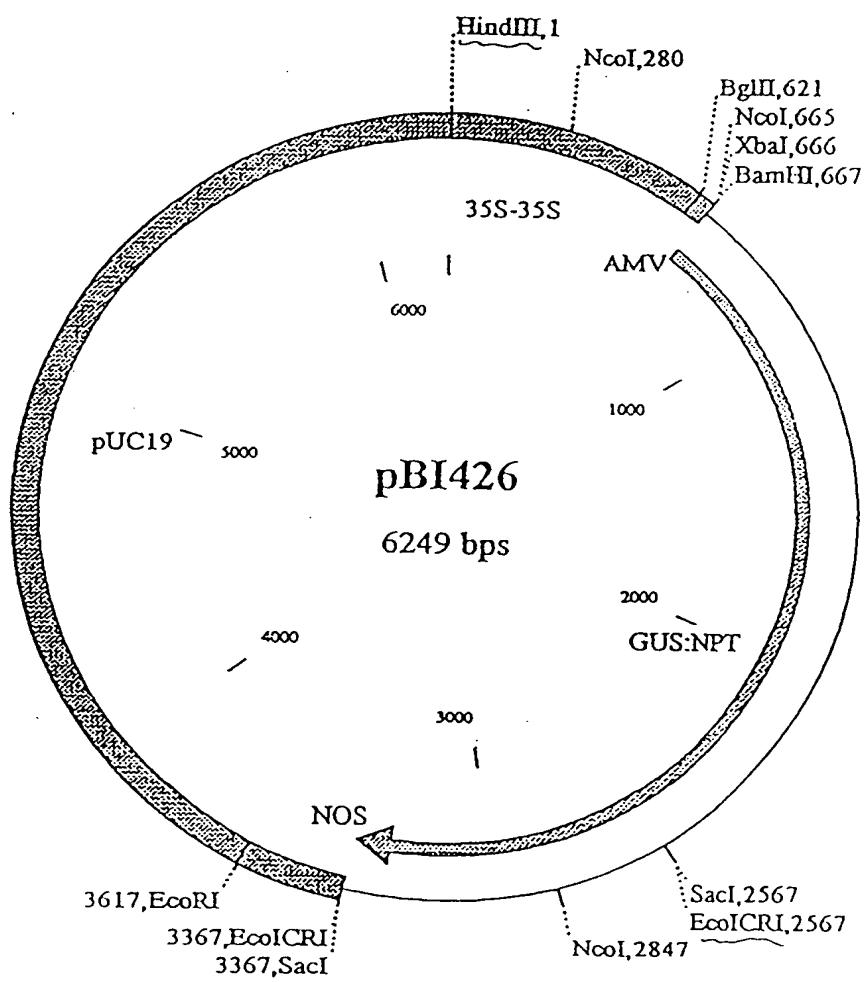


Figure 2

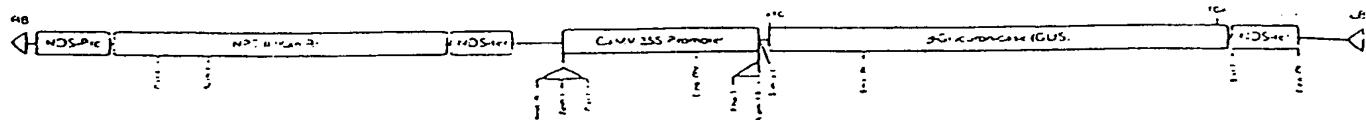


Figure 3

TATA Box
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 26 COCCCCTCGCTGCCGGACGACGAGCTCCTCCCCCTCCCGCTCGCCGCGCGCG /ataaccaccccg
 92 cgtccccctcccttttccgtttttttccgtctcgatcttgccctggatgg
 159 tggggg c gagaggcggcccgtcgccagatcggtgcgcggggggggggatcccgccggctgggg
 226 tcggcgtgcggccggatcccgtcgccggaaatggggcttcggatgtggatccgtccggccgttgcgg
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 494 gaceaatgcagcctcgtgcggagctttttag/ GTAGAAG ATG GCT GAC GCC GAG GAT
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 Gly Gly Ser Pro Gly Gly Gln Ser Phe Met

Figure 4

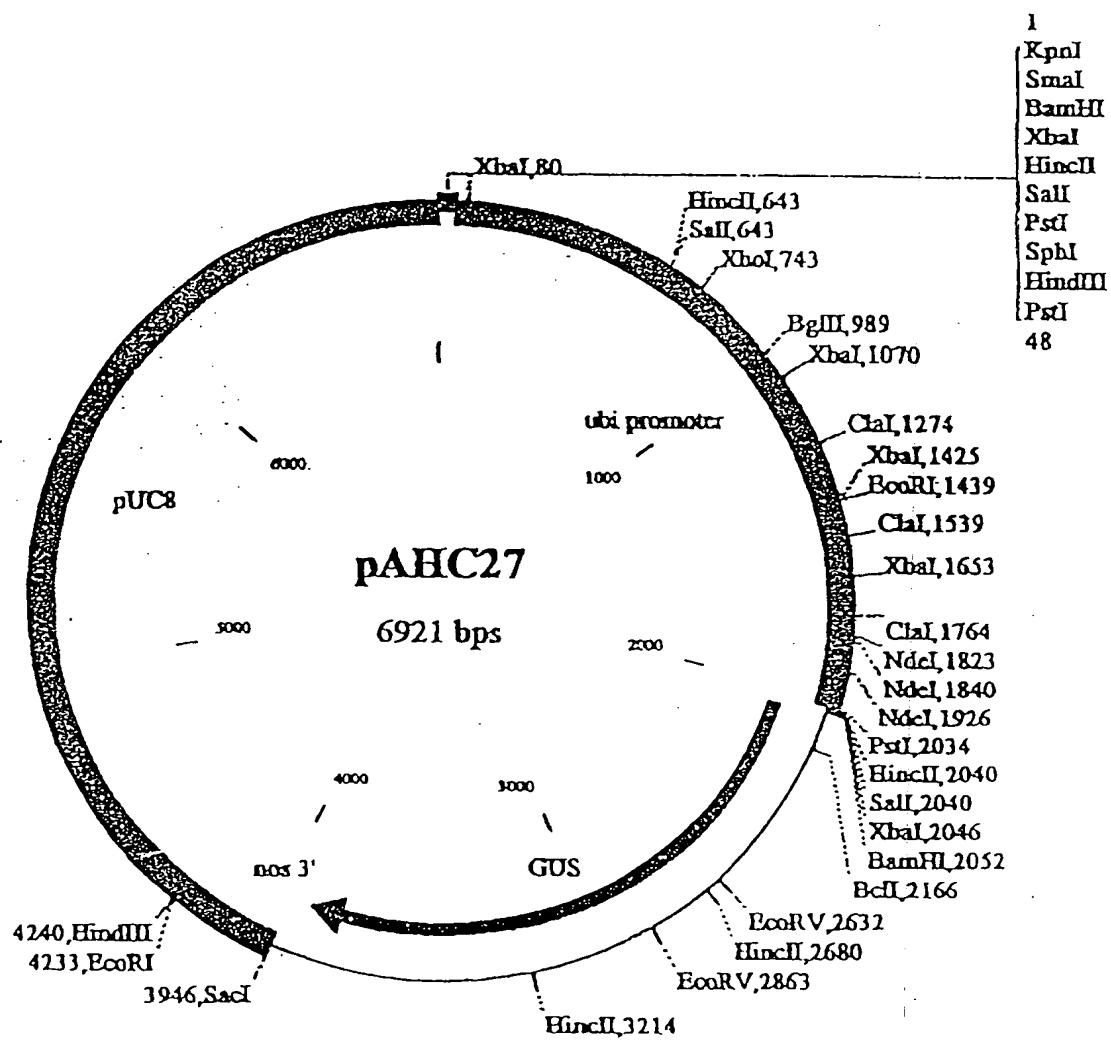


Figure 5

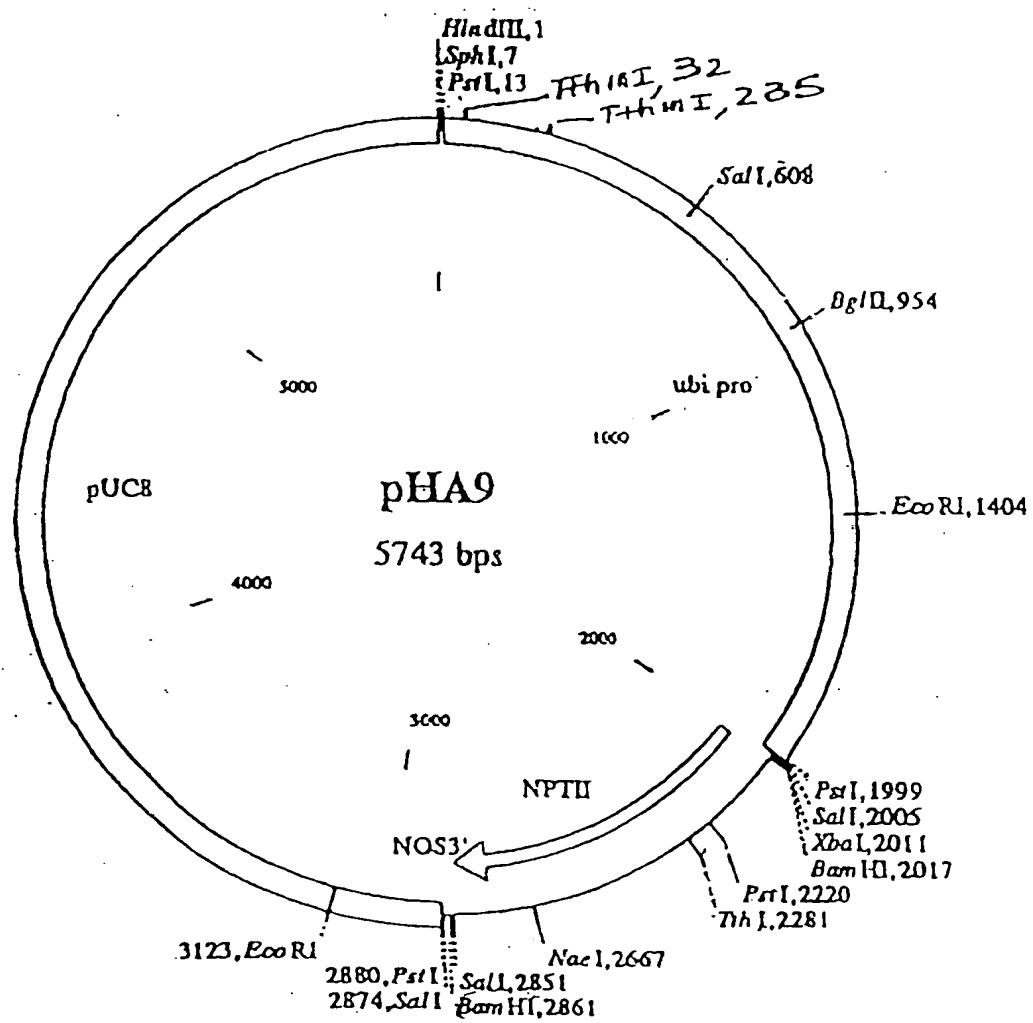
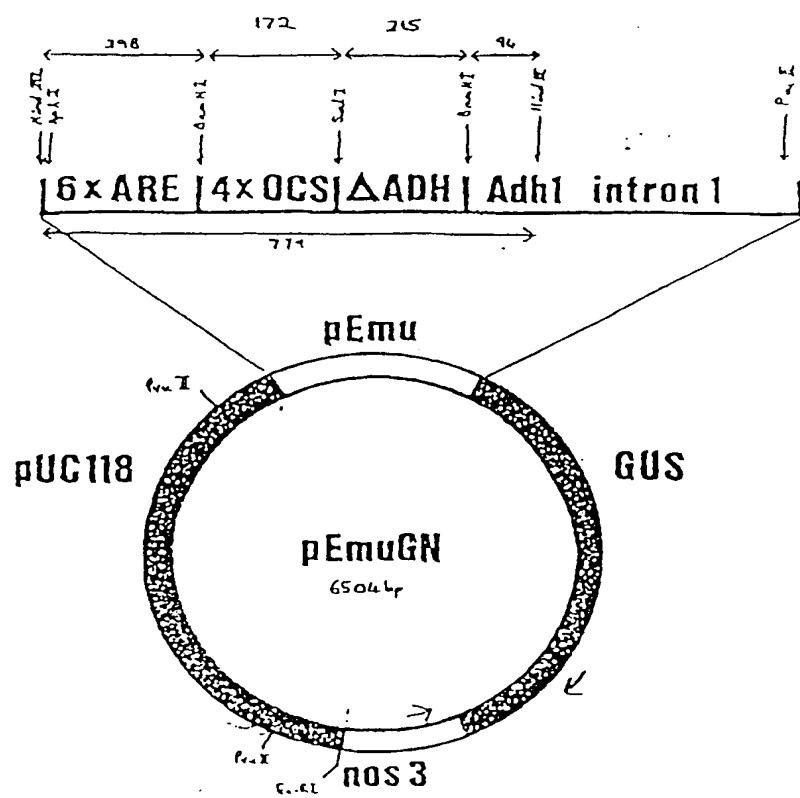


Figure 6



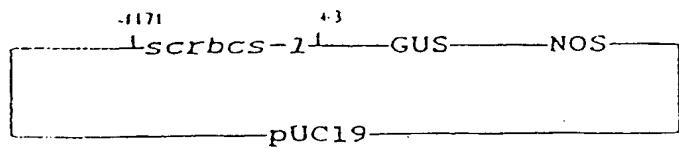


Figure 7

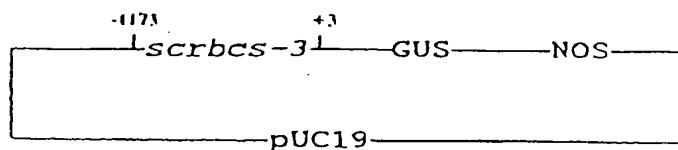


Figure 8

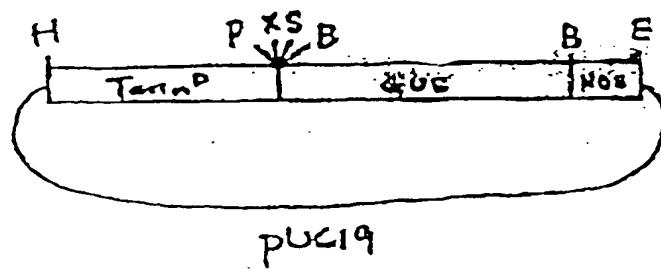


Figure 9

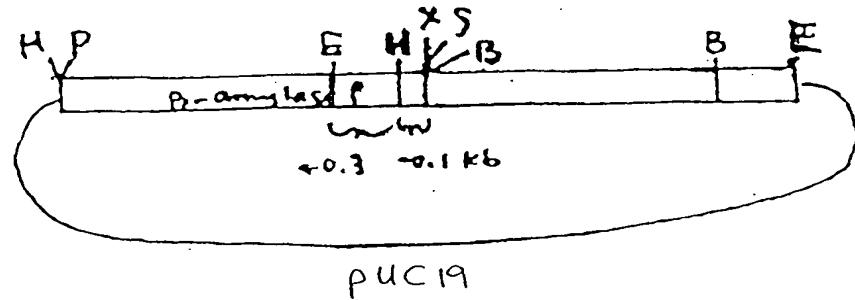


Figure 10

Figure 11

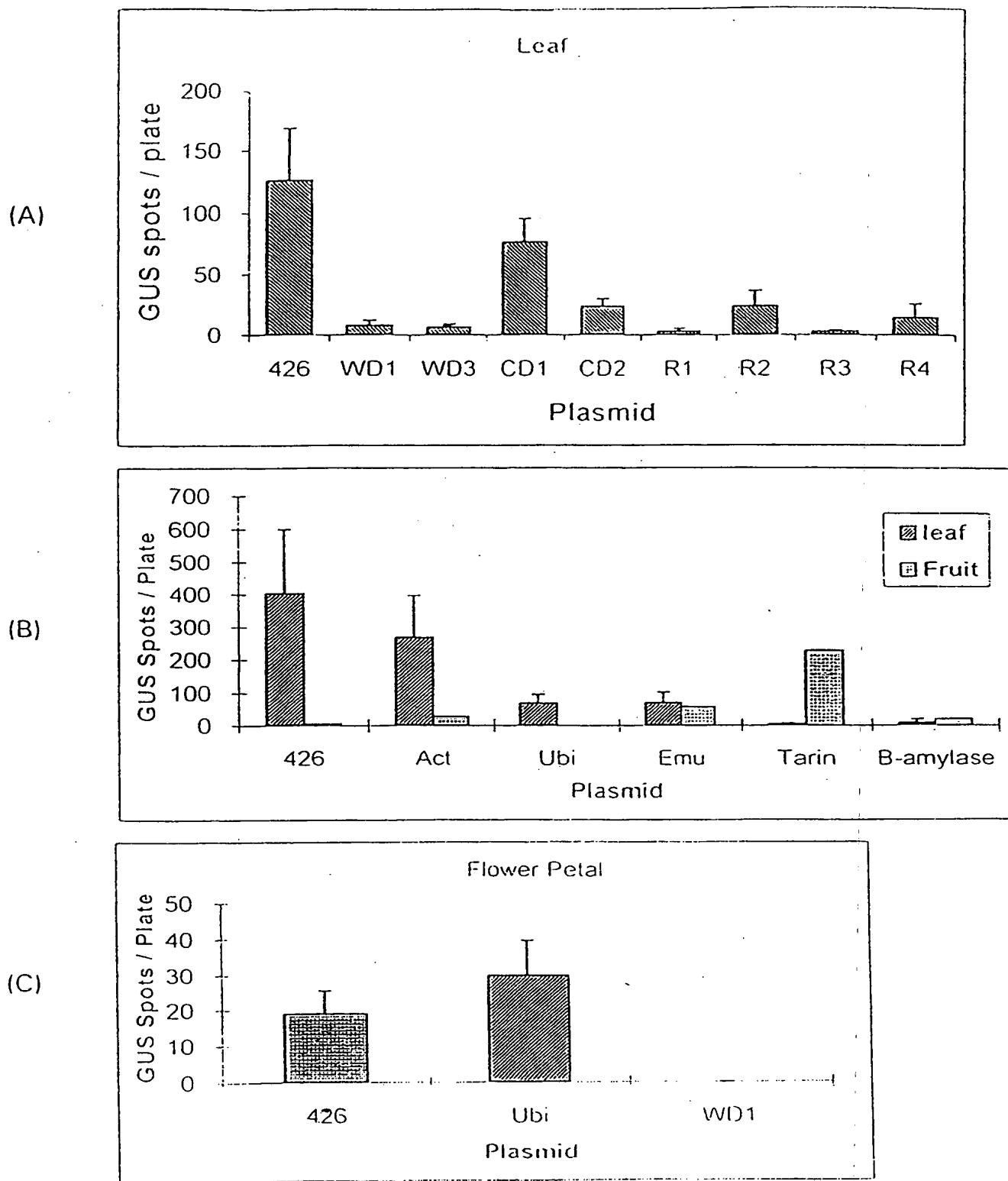


Figure 12

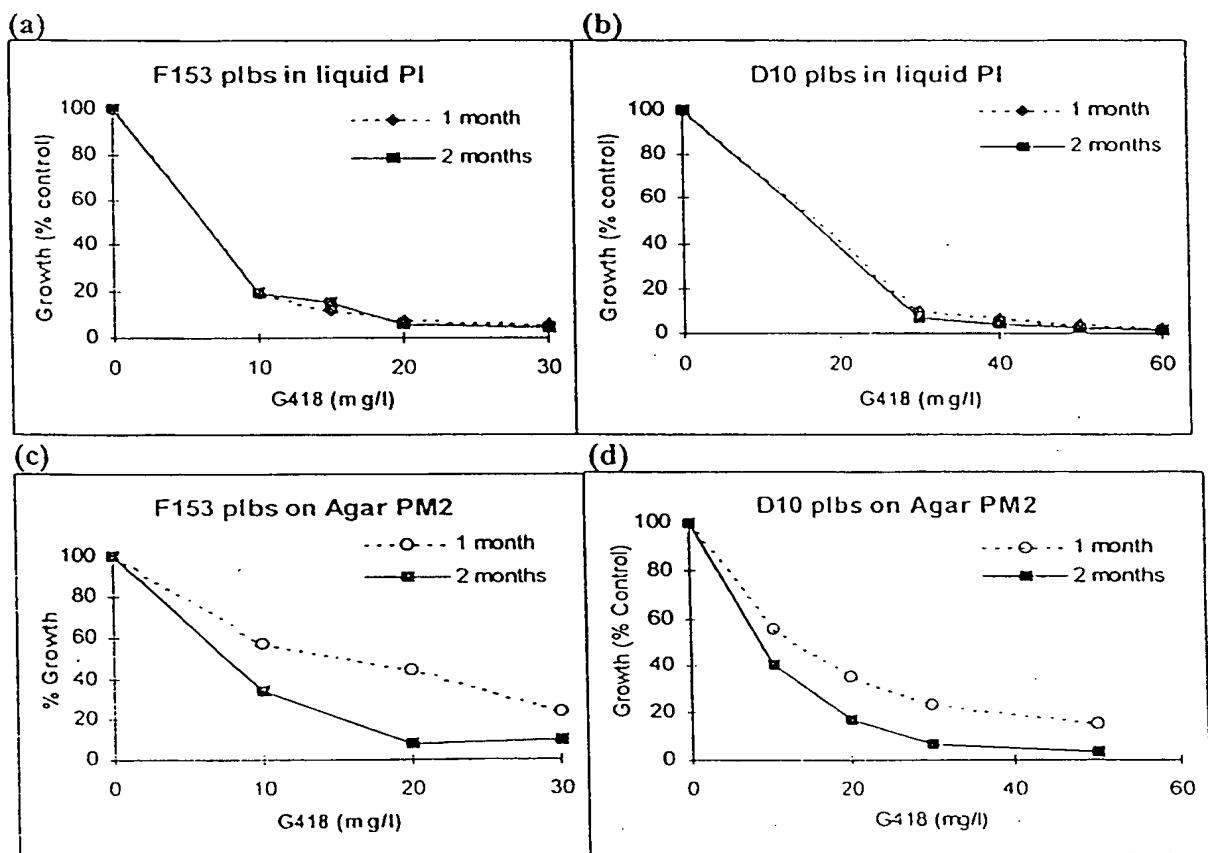


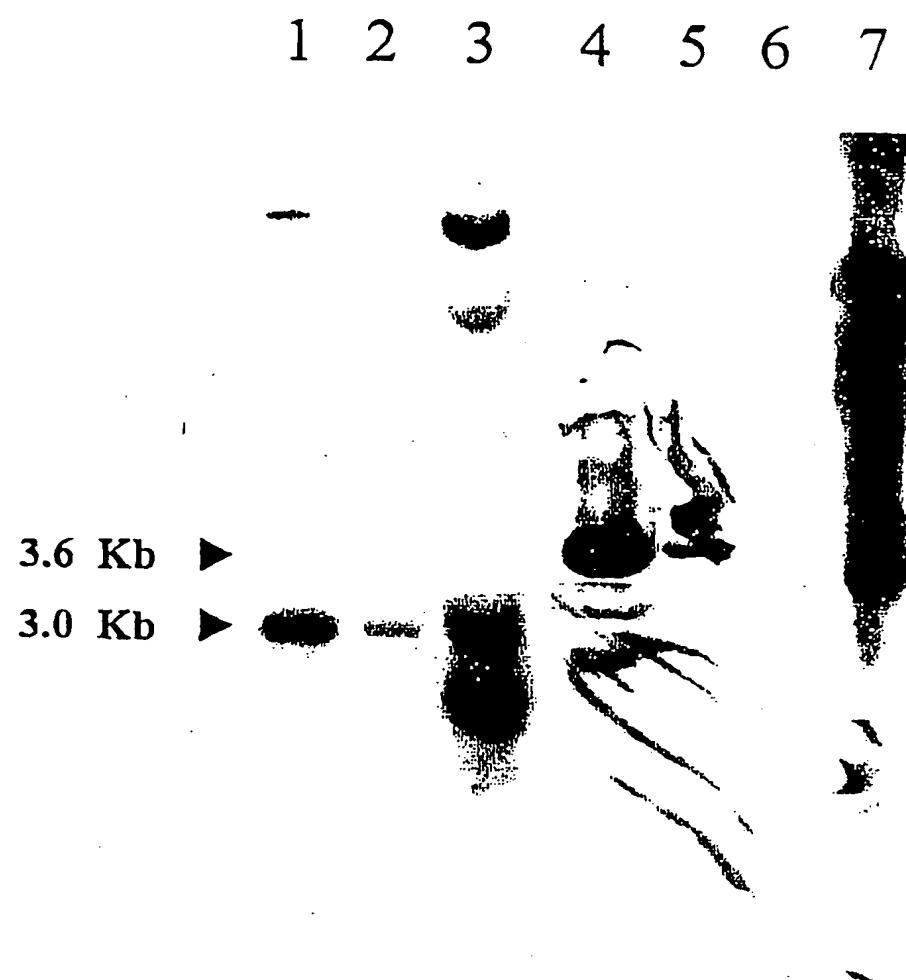
Figure 13

Figure 14

Figure 14 (Contd.)

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2521 gaagttttca gagcagtcgg atcggactcc cagatgttca agatctcagg gttcacgctg
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3481 tccatttctc aatgtatcaa tcggattc

Figure 15



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/10576

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 5/04, 15/63, 15/64, 15/82, 15/84; A01H 1/00, 1/04, 5/00

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Agricola, Caplus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PENA et al. Agrobacterium-mediated Transformation Of Sweet Orange And Regeneration Of Transgenic Plants. Plant Cell Rep. 1995, Vol. 14, pages 616-619, especially pages 616-617, see entire document.	19-23, 26
Y	McCABE et al. Direct DNA Transfer Using Electric Discharge Particle Acceleration (ACCELL Technology). Plant Cell Tiss. Org. Cult. 1993, Vol. 33, pages 227-236, especially pages 227-228, 231-233.	1-18, 24, 25, 27-32
Y	GAMBORG, O.L. Plant Cell Cultures: Nutrition And Media. in Cell Culture and Somatic Cell Genetics of Plants. 1984, Vol. 1, pages 18-26, especially pages 18-19.	1-32



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

01 JULY 1999

Date of mailing of the international search report

10 SEP 1999

Name and mailing address of the ISA/US
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/10576

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MAPES, M.O. Tissue Culture Of Bromeliads. The International Plant Propagators' Society Combined Proceedings. 1973, Vol. 23, pages 47-55, especially pages 48-50, see entire document.	2-4, 7-14, 17-27, 30-35
Y	CHIA et al. The Firefly Luciferase Gene As A Non-invasive Reporter For Dendrobium Transformation. Plant J. 1994, Vol. 6, No. 3, pages 441-446 especially page 446, see entire document.	18, 25
Y	RANGAN, T.S. Pineapple. In Handbook of Plant Cell Culture. P. K. Ammirato et al, eds. 1984, pages 373-382, especially pages 374-375, 379.	1-32
Y	ZHU et al. Isolation Of Genomic DNAs from Plants, Fungi And Bacteria Using Benzyl Chloride. Nucl. Acids Res. 1993, Vol. 21, No. 22, pages 5279-5280, see whole document.	16, 29
Y	SABELLI et al. Nucleic Acid Blotting And Hybridisation. Meth. Plant Biochem. 1993, Vol. 10, pages 79-100, see whole document.	16, 29
Y	WITTY, M. Thaumatin II: A Sweet Marker Gene for Use In Plants. Meth. Enzymol. 1992, Vol. 216, pages 441-447, especially pages 442-443.	31
Y	HAMILTON et al. Antisense Gene That Inhibits Synthesis Of The Hormone Ethylene In Transgenic Plants. Nature. 19 July 1990, Vol. 346, pages 284-287, see whole document.	32

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US99/10576**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10576

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/69.1, 410, 418, 419, 420, 430, 431, 430.1, 468, 469, 470; 800/278, 279, 283, 285, 286, 288, 293, 294, 295, 298, 301, 302

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

435/69.1, 410, 418, 419, 420, 430, 431, 430.1, 468, 469, 470; 800/278, 279, 283, 285, 286, 288, 293, 294, 295, 298, 301, 302

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claim(s)1-18, 24, 25, 27-32, drawn to a first product, a stably transformed transgenic pineapple-like totipotent body; first method of transforming a pineapple-like totipotent body via particle bombardment.

Group II, claim(s) 19-23, 26, drawn to a second method of transforming a pineapple-like totipotent body, via Agrobacterium.

Group III, claim(s) 33-35, drawn to a third method, for producing a pineapple-like totipotent body.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The special technical feature of plant transformation via particle bombardment is not shared nor required by the other groups. The special technical feature of plant transformation via Agrobacterium is not shared nor required by the other groups. The invention of group three does not share nor require the transformation methods of the other groups.